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Date of Signature and Deposit: 12-28, 2006

Sara D. Vinarov

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Robert G. Lowery
John Majer

Date: Dec 22, 2006

Serial No.: 10/769,578

Group Art Unit: 1637

Filed: 01/30/2004

Examiner: Mark Staples

Title: ASSAY METHOD FOR GROUP TRANSFER REACTIONS

File No.: 112520.00004

DECLARATION OF ROBERT LOWERY
Under 37 CFR 1.132

Mail Stop Amendment
Commissioner for Patents
P O Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Robert G. Lowery, on oath declare and sayeth that:

1. I am the same Robert G. Lowery who is a named co-inventor on the above-identified patent application. I make this declaration in support of that patent application. I am currently employed by BellBrook Labs LLC as President and CEO. I have worked as a researcher specializing in the general area of biochemistry for 17 years. I have published extensively in this area. A copy of my *Curriculum Vitae* is attached as Exhibit A.

2. I have reviewed the above-identified application and understand the nature and scope of the invention claimed therein. I have also reviewed the Office Action issued by the U.S. Patent and Trademark Office (USPTO) on October 2, 2006 for this application. I understand that currently Claims 1-3, 7-10, 12-15, 19-24, 28 and 28 stand rejected as being obvious under 35 U.S.C. 103(a). This Declaration is submitted to show the claimed process and products are commercially successful

and this commercial success is directly attributable to the process and products defined by the claims.

3. At the outset, in my experience a generic group transfer reaction assay with related products, which enables detection of donor-products with high efficiency and sensitivity is of high value to the pharmaceutical industry. This is one of the reasons why the claimed invention is commercially successful. Since November of 2005, when we first introduced one embodiment of the invention - the Transcreener™ Kinase Assay - into the market place, we made repeat sales to all of the twenty largest pharmaceutical companies, and total sales are approaching $1M. This is the highest first year sales for any HTS product that I am aware of from my ten years of experience at three different companies.

4. Furthermore, there is strong interest from other companies in licensing our commercialized process and product, namely the Transcreener™ Kinase Assay. Three of the major suppliers of HTS assay products – all of them much larger than BellBrook Labs – have inquired repeatedly about licensing the Transcreener™ Kinase Assay technology. We are currently in negotiations with two of these companies regarding licensing fees and royalty payments. It is certain that these companies performed extensive due diligence on the intellectual property in this area before approaching us for out-licensing. Their willingness to consider paying licensing fees and royalties for the technology indicates that they consider the claimed methods and products to be novel, non-obvious and extremely useful.

5. I believe the reason for this outstanding record of commercial success is that our claimed assay for detection of a donor-product for a group transfer reaction fulfills a long felt need in the pharmaceutical industry. It is noted that because of the success with the Transcreener™ Kinase Assay, the examples that I will discuss herein will relate to Kinases. However, I believe the statements made here in are equally applicable to the other group-transfer reactions claimed in our application.

6. To begin with, more than one third of the total drug discovery effort worldwide is focused on protein kinases, and there is a very strong push within the industry to screen more of the 518 human protein kinases, more rapidly. There is also increasing interest in screening non-protein kinases, including lipid and carbohydrate kinases, which number more than 150 distinct proteins. A major technical hurdle to moving more kinases into HTS has been the lack of robust generic assay

methods. There are more than a dozen kinase assay methods in use for HTS that are sold by about twenty different vendors; most are not generic. Researchers rely on detection of a phosphopeptide product using antibody or a metal chelate complex. These methods require development of new reagents for each kinase, or for subsets of kinases that use a common peptide acceptor. The use of a generic assay method applicable to all 518 human protein kinases can potentially save millions of dollars in assay development, and greatly simplify selectivity profiling of candidate molecules across multiple kinases. Moreover, the phosphopeptide assay methods are not applicable to lipid or carbohydrate kinases, and thus targets in these families have been even more problematic for screeners in the pharmaceutical companies.

7. Furthermore, utilizing ADP detection for a generic kinase assay and detecting nucleotides with antibodies were concepts known in the literature prior to the invention. However, use of antibodies for detecting a donor-product, such as ADP in a kinase reaction was unprecedented before the claimed invention. There is good reason for this – mostly having to do with the perceived difficulty of developing antibodies with the required properties for the invention. Therefore, despite the high value of the invention, no one was previously motivated to combine the elements to produce the claimed methods and products.

8. To my knowledge there were two generic kinase assay methods available prior to our invention. Both, however, relied on a coupled enzyme assay format in which ADP or ATP are quantifed after first converting them to something else that can be readily detected with one or more "coupling enzymes". Examples of these assays are the "ADP Quest" assay sold by DiscoveRx and the "Kinase-Glo" assay sold by Promega. The method of our claimed invention, <u>direct immunodetection of a donor-product,</u> suitably ADP, is clearly preferable to either of these approaches because it is not subject to interference of test compounds with coupling enzymes. Such interference is relatively common and it obfuscates the results of inhibitor screens by causing false positives or false negatives. The method of our claimed invention, <u>direct immunodetection</u> of ADP does not suffer this major drawback of the other generic methods, and so its value is quickly being recognized by the multi-billion dollar pharma industry.

9. The generic nature of the method is especially valuable in applications involving the screening of diverse kinases; i.e., situations that would require the use of several different non-generic methods. For example, one of the ten largest pharmaceutical companies has purchased

more than $200,000 of Trancreener Kinase Assay kits and has done extensive validation of the assay on a panel of fifteen different kinases. They are so impressed with its advantages over other methods that they have convinced one of the major HTS assay service providers to adopt the Transcreener Kinase Assay for their kinase profiling service. This pharma company has essentially promised the service provider more than $1M of business in the coming year if they use the Transcreener Kinase Assay for their kinase profiling.

10. Given the importance of group transfer reactions, namely kinases, it would appear that all of the obvious ideas relating to kinase assays would have been put to practice some time ago by researchers in this industry. Despite the obvious value of generic assay methods and the clear advantages of detecting ADP directly with an antibody rather than indirectly via interconversion by additional enzymes (lower costs, less chance for interference, higher accuracy), there are no literature reports proposing immunodetection of ADP as a kinase assay method nor have any companies put the method into practice.

11. I believe there were many reasons for the failure of the industry to develop assay methods that <u>directly detect donor-product</u>. One reason was the perceived inability to <u>directly detect donor-product</u>, such as ADP in the presence of ATP. In this regard, a major hurdle was the development of a suitable antibody for recognizing a donor-product, such as ADP with sufficient selectivity over ATP.

12. I believe that most people with ordinary skill in the art were not aware that antibodies against nucleotides even existed at the time our application was filed. Antibodies to proteins and peptides, and even to small molecules such as hormones were widely known, but antibodies to nucleotides were not known well. This is because there are very few recent publications and very few commercial products relating to antibodies against nucleotides . Even now, when confronted with the possibility of developing antibodies against ADP, most researchers express concern about the possibility of autoimmunity issues and phosphodiester bond lability. Also, the literature, most of which is at least twenty years old, describes efforts to develop antibodies that recognize DNA or specific bases in DNA, including mutated residues. There is very little on using antibodies for detection of mononucleotides. In fact, I believe development of antibodies that discriminate with high selectivity between donor-product , such as ADP and donor molecule, such as ATP (on the basis of a single phosphate) would have been considered unrealistic.

13. Next, there was a general perception among those practicing in the field (including myself) that antibodies with exquisitely high selectivity – on the order of 1,000-fold – would be required for the invention. The development of such antibodies was thought by experts – including an NIH review panel - to be a dubious undertaking. In fact, in reviewing a Phase I grant submitted by me on December 1, 2004 entitled "Generic Fluorescent HTS Assay for Protein Kinases," the reviewers stated the following in reference to an antibody generated to an ADP-ribose antigen (Bredehorst et al., 1978) that cross reacted with various adenine nucleotides:

> "Unfortunately, its specificity (for AMP) versus ADP, which is the most relevant comparison, was only 40-fold. This is significantly less than their (the P.I.'s) target of 1000-fold. Because the relative difference between one phosphate group and two is likely, in terms of antigenicity, more than the difference between two phosphate groups and three, there is only little confidence that the required antibody (for ADP) would be feasible."

Not surprisingly, the enthusiasm of the review panel for the proposed development of the assay was relatively low and the grant application under review was not funded. (Note: Bredehorst et al. cited in the NIH review is the same document cited by the Examiner in the present Office Action.) However, contrary to the comments of the NIH review panel, applicants discovered that much lower selectivity – approximately 100-fold – was sufficient for the assay to be effective. Thus, even an NIH review panel thought the selective immunodetection of a donor-product, such as ADP in the presence of excess ATP was not a scientific feasible idea. This argues strongly against obviousness to those with ordinary skill in the art.

14. Next, I am unaware of any document demonstrating how to successfully develop antibodies against an ADP antigen or any other nucleotide containing a pyrophosphate linkage. In fact, I declare the literature actually teaches against the development of such antibodies. The Examiner cited Bredehorst et al. as providing a teaching for development of antibodies to ADP. Bredehorst attempted to raise antibodies to an ADP-ribose antigen, and fortuitously observed a low level of crossreactivity to ADP. However, he concluded that most of the ADP-ribose was hydrolyzed to AMP in the rabbit's serum, resulting in antiserum that bound predominantly to AMP. I conclude from these results that like ADP-ribose, ADP would be hydrolyzed at the pyrophosphate bond to AMP, resulting in little antibody to ADP. Additionally, one with skill in the art investigating this approach would conclude that ADP is even more labile than ADP-ribose in the serum of a rabbit or mouse because of the presence on the surface of platelets of nucleotidases that dephosphorylate ATP and ADP (See: Koszalka, P., et al., Targeted disruption of cd73/ecto-5'-

nucleotidase alters thromboregulation and augments vascular inflammatory response. *Circ Res,* (2004) 95(8): p. 814-21; and Nishio, H., et al., Effect of concanavalin A on 5'-nucleotidase activity of rabbit blood platelets. *Jpn J Pharmacol*, (1987) 43(2): p. 230-3).

15. Literature describing the synthesis of non-hydrolyzable nucleotide analogs for use as antigens further bolstered the belief of researchers that the lability of pyrophospate linkages would prevent their inclusion in antigens. Nine years after Bredehorst was published, the senior author on that paper published on the synthesis of an ADP-ribose analog with a carbon atom replacing oxygen in the pyrophosphate linkage, rendering it resistant to enzymatic degradation (Meyer, T. and H. Hilz, Production of anti-(ADP-ribose) antibodies with the aid of a dinucleotide-pyrophosphatase-resistant hapten and their application for the detection of mono(ADP-ribosyl)ated polypeptides. *Eur J Biochem*, (1986) 155(1): p. 157-65). The resulting antibodies bound ADP-ribose approximately four-fold more tightly than AMP, significantly improving on the earlier effort of Bredehorst et al. with unmodified ADP-ribose and supporting the hypothesis regarding lability of the pyrophosphate linkage.

16. In a more recent and closely related example, investigators developing an antibody to dideoxy-ATP used an antigen with a citrate moiety replacing the triphosphate portion of the molecule (Le Saint, C., et al., Determination of ddATP levels in human immunodeficiency virus-infected patients treated with dideoxyinosine. *Antimicrob Agents Chemother*, (2004) 48(2): p. 589-95). Their rationale for this structural modification of ddATP was clearly stated: "The development of antibodies with directly polyphosphate nucleoside molecules as haptens is a difficult task because the hapten phosphodiester bonds are rapidly hydrolyzed by endogenous phosphatases." This document was submitted for publication in April of 2003, shortly after the provisional patent application for the claimed invention was filed. Thus, it cannot be considered as relevant art, but indicates the general consensus of those with skill in the art regarding the lability of pyrophosphate linkages. This consensus significantly weakens one's motivation to pursue immunodetection of ADP as a kinase assay method at the time the invention was filed.

17. In addition to the lack of documents describing antibodies to ADP, there was no commercial sources for such antibodies, despite their clear value until applicants' discovery. Since negative data is often not published, commercial availability is often a good indicator of the feasibility of developing a particular antibody if there is value in it, one of the many companies that

produce antibodies would provide it. Moreover, expertise on generating antibodies against challenging antigens is concentrated in the commercial antibody vendors, since they do the bulk of the antibody work for academic researchers.

18. In sum, I declare the evidence provides - (1) the lack of documents describing the development of antibodies to ADP or any other diphospho-nucleotide antigens; (2) the unavailability of such antibodies from commercial providers; and (3) the general consensus of researchers with expertise on the development of anti-nucleotide antibodies that an antigen with a pyrophosphate linkage would be degraded - shows the development of antibodies to ADP was not something that one with ordinary skill in the art would have been motivated to pursue and thus, was not obvious.

19. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Further declarant sayeth not.

Dated: Nov 22, 2006

Robert G. Lowery

EXHIBIT A

CURRICULUM VITAE

Robert G. Lowery, Ph.D.
301 Wilderness Way
Brooklyn WI, 53521
608-455-2617 (home)
Bob.Lowery@Bellbrooklabs.com

PERSONAL DATA

Born December 17, 1956
Social Security no. 566-84-2894
Married, four children

EDUCATION

Bachelor of Arts in Biochemistry, University of California, Berkeley (1980)
Ph.D. in Biochemistry, Enzymology of Nitrogen Fixation, University of Wisconsin - Madison (1988)
Postdoctoral Studies, Genetics of Pyridine Nucleotide Metabolism, University of Utah (1989)

PROFESSIONAL EXPERIENCE

May 2002 to present:

President and Founder
BellBrook Labs, LLC
Madison, Wisconsin

A research company focused on developing biological assay systems and computational methods that accurately model human disease processes, thereby enabling more rational drug discovery.

November 1992 to May 2002:

PanVera Corporation
Madison, Wisconsin

Director, Product Development (11/92 – 6/99)

Vice President, Research and Development (6/99-5/02)

Played a key role in establishing PanVera's scientific and commercial focus since its inception in 1992. PanVera introduced the concept of a complete reagent package for HTS drug discovery assays: combining purified drug target proteins, fluorescent probes and validated assay protocols that can be used in an automated HTS environment. Along with the PanVera's scientific cofounder, I built a strong R&D and manufacturing team around this concept, enabling rapid growth to a profitable company of over 100 employees with revenues in excess of $20 million.

May 1989 to November 1992:

Promega Corporation
Madison, Wisconsin

Director of Production (April 1992 to November 1992)
Group Leader, Modifying Enzymes Group (January 1990 to April 1992)

Production Scientist, Modifying Enzymes Group (May 1989-December 1989)

GRANTS and AWARDS

Academic

NIH National Service Award (1983-1986)
Steenbock Departmental Fellowship (1986-1987)
American Cancer Society Postdoctoral Fellowship (1988-1989)

Industrial (recent)

National Institute of General Medical Sciences, Phase II SBIR Award, $1,149,954 (2002); High Throughput Assays for Drug Glucuronidation

National Institute of General Medical Sciences, Phase I SBIR Award, $208,000 (2003); Fluorescent HTS Assays for Human Sulfotransferases

National Institute of General Medical Sciences, Phase II SBIR Award, $1,028,000 (2005); Fluorescent HTS Assays for Human Sulfotransferases

National Cancer Institute, Phase I SBIR Award, $173,000 (2005); Generic Fluorescent HTS Assay for Protein Kinases

National Institute of Dental and Craniofacial Research, $252,748 (2005); Microfluidic Reconstituted Mammary Tissue System

National Cancer Institute, Phase II SBIR Award, $648,000 (2006); Generic Fluorescent HTS Assay for Protein Kinases

National Institute of General Medical Sciences, Phase I SBIR Award, $163,177 (2006); Fluorescent HTS Assays for Methyltransferases in Neurodegenerative Diseases

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Burke, Thomas J.; Lowery, Robert G.; Kleman-Leyer, Karen. Identification of a protein carrying an N-terminal polyhistidine affinity tag (2003) U.S. Patent Application #20030044789

Burke, Thomas J.; Bolger, Randall E.; Ervin, Kerry M.; Lowery, Robert G.; Checovich, William J. Method for quantifying competitive binding of molecules to proteins utilizing fluorescence polarization (2003) U.S. Patent #6,511,815

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Lowery, Robert G., C.L. Chang, L.C. Davis, P.W. Stevens, M.C. McKenna, and P.W. Ludden. (1989) Substitution of hsitidine for arginine 101 of dinitrogenase reductase disrupts electron transfer to dinitrogenase. Biochemistry, 28; 1206-1212.

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